ToxStrategies

Innovative solutions
Sound science

Technical Comments on Portions of the Environmental Protection Agency's (EPA's) Draft IRIS Toxicological Review of Formaldehyde (Inhalation)

Prepared by: Chad Thompson, PhD
Senior Consultant
ToxStrategies, Inc.
23501 Cinco Ranch Blvd.
Suite B226
Katy, TX 77494

Submitted to: Docket ID # EPA-HQ-ORD-2010-0396 for the Formaldehyde (Inhalation) IRIS Assessment via www.regulations.gov

Submitted on: 13 June 2022

Overview

Pertinent to the release of the US EPA's External Review Draft of *Toxicological Review of Formaldehyde-Inhalation* I was surprised to see that a 2020 review article I coauthored on the mode of action of formaldehyde-induced nasal tumors in *Critical Reviews in Toxicology*[1] was not cited anywhere in the 193-page *Assessment Overview*, 789-page *Toxicological Review*, or the 1058-page *Supplemental Information* documents[2]. This omission by the U.S. EPA is unfortunate as there are critical data integration topics in the review article that might have informed U.S. EPA's assessment. Moreover, Thompson et al. (2020) is an update of the mode of action (MOA) for formaldehyde-induced nasal tumors published by McGregor et al. (2006), which the U.S. EPA did cite in their assessment. As such, the omission of Thompson et al. (2020) is disappointing.

It should also be noted that I worked on earlier versions of U.S. EPA's risk assessment for formaldehyde in 2003 as an American Association for the Advancement of Science Risk Policy Fellow serving at U.S. EPA ORD NCEA in Washington and thereafter as a staff scientist at the same agency from 2004-2009. As will be shown in my comments, some of the mechanistic insights just coming to light in 2003 have matured in the intervening decades and intersected with groundbreaking formaldehyde research on the dosimetry of inhaled formaldehyde. As will be shown, the Thompson et al. (2020) article provides important data integration information that the U.S. EPA failed to conduct in

1 Thompson et al. (2020) An updated mode of action and human relevance framework evaluation for formaldehyde-related nasal tumors. Critical Reviews in Toxicology. 50(10): 919-952.
2 Thompson et al. (2020) was written with support from the ACC, as is my review of US EPA's 2022 draft assessment.

ED_014350_00001047-00001

their assessment. While some of the critical studies related to understanding the MOA for nasal tumors are cited, the U.S. EPA seemed to take away only the most superficial findings from these studies rather than integrate data streams to inform the MOA of formaldehyde-induced nasal tumors.

Below are comments relevant to the missing Thompson et al. (2020) article (and others), additional comments relevant to the MOA evaluation for formaldehyde-induced nasal tumors, as well as separate comments related to some of the noncancer evaluation in the U.S. EPA (2022) assessment. Broadly speaking, these comments highlight the following:

1. Failure to integrate relevant science into the MOA for nasal tumors in rodents
2. Failure to response appropriately to previous review by the National Research Council
3. Failure to adhere to U.S. EPA MOA guidance and practices
4. Failure to consider nonlinear approaches for protecting against upper respiratory tract cancers
5. Failure to use appropriate benchmark dose modeling software and practices
6. Poor organization of the various assessment documents

Main Comments

1. U.S. EPA's literature search and/or review of their literature search results incorrectly filtered out relevant MOA evaluations that the agency should have considered.

Appendix F indicates that a draft formaldehyde assessment was suspended in 2017 and subsequently unsuspended in 2021. Appendix F also indicates that an updated literature search was conducted spanning from January 2016 to May 2021. The stated purpose of the search was to identify new epidemiological, toxicological, and mechanistic studies, as well as "tag" secondary literature (i.e., non-primary research). It is unclear what literature was "tagged" and subsequently processed.

The following review articles relevant to the MOA of formaldehyde and thus the U.S. EPA risk assessment are not cited anywhere in the *Toxicological Review, Assessment Overview*, or *Supplemental Information*. It is unclear if these articles were not captured in U.S. EPA's literature searches or whether they were captured but intentionally discarded by the U.S. EPA:

> Thompson, CM, Gentry, R, Fitch, S, Lu, K, Clewell, HJ (2020) An updated mode of action and human relevance framework evaluation for Formaldehyde-Related nasal tumors. Critical Reviews in Toxicology 50(10): 919-952.
> **(Available online 02/18/2021; 1046 views as of 05/23/2022)**
>
> Gentry, R, Thompson, CM, Franzen, A, Salley, J, Albertini, R, Lu, K, Green, T (2020) Using mechanistic information to support evidence integration and

synthesis: a case study with inhaled formaldehyde and leukemia. Critical Reviews in Toxicology 50(10): 885-918.
(Available online 02/04/2021; 1333 views as of 05/23/2022)

Andersen, ME, Gentry, R, Swenberg, JA, Mundt, KA, White, KW, Thompson, C, Bus, J, Sherman, JH, Greim, H, Bolt, H, Marsh, GM, Checkoway, H, Coggon, D, Clewell, HJ (2019) Considerations for refining the risk assessment process for formaldehyde: Results from an interdisciplinary workshop. Regulatory Toxicology and Pharmacology 106: 210-223.

Thompson, CM (2018) Commentary on new formaldehyde studies in Trp53 haploinsufficent mice: further support for nonlinear risk from inhaled formaldehyde. Dose-Response April-June 2018:1-2.

U.S. EPA should consider the information included in these publications and revise its assessment accordingly.

2. U.S. EPA failed to integrate two highly relevant data streams into their MOA analysis.

I began working on the toxicity of formaldehyde in 2003 while working at the U.S. EPA. At that time, it had recently been discovered that formaldehyde dehydrogenase played a dual role in regulating cellular formaldehyde and nitrosoglutathione (GSNO) levels. Due in large part to the interest in the latter function, mice deficient in this enzyme were engineered to study the role of nitrosothiol status in certain diseases. Due to this dual function, the enzyme is known as both formaldehyde dehydrogenase and GSNO reductase (GSNOR) but is officially named alcohol dehydrogenase-5 (ADH5) in humans. By knocking out ADH5 in mice, the ability to detoxify endogenous and exogenous formaldehyde would be expected to be compromised and therefore potentiate any formaldehyde-related toxicity or genotoxicity. As will be seen, studies in ADH5 null mice provide valuable insight into the MOA of formaldehyde and were given little attention in the U.S. EPA assessment.

As studies in ADH5 null mice proceeded, a separate line of work from Dr. Jim Swenberg's group at UNC developed a dual isotope labelling method that allowed for the measurement and discernment of endogenous and exogenous formaldehyde-DNA adducts. This work allowed for the quantification the endogenous background levels of formaldehyde-DNA adducts in different tissues as well as quantification of the burden of exogenous formaldehyde-DNA adducts following inhalation exposure to dual labeled formaldehyde. As summarized graphically in Thompson et al. (2020), inhalation studies in rats indicate that exposure to $\leq$2 ppm formaldehyde results in exogenous formaldehyde-DNA adducts well below the endogenous level. Adduct levels approach endogenous levels following exposure to 6-10 ppm, whereas adduct levels exceed endogenous levels following exposure to 15 ppm formaldehyde. Despite these increases in adduct levels, measures of mutagenic and clastogenic DNA damage in nasal tissue of rats exposed up to 15 ppm formaldehyde are negative—suggesting that the observed

increases in formaldehyde-DNA adducts are not genotoxic. A likely interpretation is that given the ubiquitous presence of endogenous formaldehyde, most cells are capable of handling more formaldehyde than is typically present, i.e., there is functional reserve.

Over the past few years, these disparate lines of research on ADH5 null mice and measurement of endogenous and exogenous formaldehyde-DNA adducts have intersected to provide insight into the MOA of formaldehyde. As discussed in Thompson et al. (2020), studies in ADH5 null mice indicate that such mice have ~2-fold higher levels of endogenous formaldehyde-DNA adducts compared to wild type mice with functioning ADH5 (Pontel et al. 2015). Critically, these mice did not demonstrate increased markers of genotoxicity compared to wild type mice. However, mice engineered with loss of both ADH5 and the Fancd2 DNA repair enzyme did exhibit increased markers of DNA damage. One conclusion from these studies is that a 2-fold increase in formaldehyde-DNA damage does not result in genotoxicity in cells that have component repair mechanisms. It should be noted that OECD *in vivo* genotoxicity testing guidelines use wild type animals as opposed to animals engineered to be susceptible to the agents being tested. Stated differently, one should not interpret the genotoxicity observed in the dual knockout mice as evidence that formaldehyde is genotoxic when, in fact, a 2-fold increase in formaldehyde-DNA adducts was not genotoxic in animals with intact DNA repair systems. Indeed, as discussed in Thompson et al. (2020), Big Blue mice (harboring transgenes to detect DNA mutations) crossed with ADH5 null mice have the same background mutant frequency as ADH5-competent Big Blue mice (Leung et al. 2013). Assuming these Big Blue ADH5 null mice have twice the endogenous formaldehyde-DNA adducts, the presumed increase does not appear to result in higher rates of background mutation. As previously mentioned, exposure to 2 ppm formaldehyde results in exogenous adducts below endogenous levels, i.e., does not increase formaldehyde-DNA adduct burden 2-fold. Together, these data begin to define a threshold where inhaled formaldehyde cannot be considered genotoxic, except perhaps to those that are severely compromised due to genetic predispositions. Notably, the U.S. EPA did not cite Leung et al. (2003) in their assessment. Pontel et al. (2015) was cited three times in the *Toxicological Review*, where it appears mainly to be cited in passing as support for potentially susceptible individuals as opposed to integrating data streams to inform the genotoxic potential of formaldehyde and related MOAs.

One might argue that the above data, being integrated from disparate studies investigating different scientific questions, are not compelling enough to inform the MOA. However, as discussed in Thompson et al. (2020), a recent study by Dingler et al. (2020) provides further support integrating these data to inform MOA. Specifically, Dingler et al. (2020) reported that ADH5 null mice exhibit 2-fold higher serum formaldehyde levels and 5-fold increases in formaldehyde-DNA adduct levels in kidney, liver, and brain yet did not exhibit increases in blood micronuclei or mutations in the aforementioned tissues. However, dual ADH5 and aldehyde dehydrogenase-2 (ALDH2) null mice exhibited 11-fold increases in serum formaldehyde levels and ~20-fold increases in formaldehyde-DNA adducts in kidney, liver, and brain. These mice exhibited significant increases in blood micronuclei, and DNA damage in tissues (base pair mutations, deletions, insertions). This study indicates that formaldehyde-induced DNA damage occurs when

the formaldehyde-DNA adduct levels are somewhere between 5 to 20-fold higher than normal. Based on the dual labeling inhalation studies from the Swenberg lab, such combined levels of endogenous and exogenous adducts might occur at 15 ppm and higher. One could also argue that liver, kidney, and brain cells are slowly dividing cells and thus the increased proliferation observed in nasal tissue might render lower fold increases in formaldehyde-DNA adducts as genotoxic. Even so, the totality of the data suggest that exposures above 2 ppm are needed to increase formaldehyde-DNA adducts *and* cell proliferation. These data clearly support a threshold in carcinogenic action of formaldehyde. Notably, Dingler et al. (2020) was cited twice in the *Toxicological Review*, each time along with Pontel et al. (2015) and each time in the context of support for potentially susceptible individuals. Nowhere was there an attempt to integrate the ADH5, adduct, and genotoxicity data into the MOA as summarized above and in Thompson et al. (2020). In fact, Dingler et al. (2020) was included in Table F-11 "Mechanistic studies relating to respiratory or systemic inflammatory and immune responses" in a subsection of the table titled "Models, Endogenous Formaldehyde, or Other Studies" of the *Supplemental Information*. Therein, Dingler et al. (2020) is described as "Possibly impactful" based on the following rationale:

> "Serves as included reference study for discussion of potential sources of susceptibility relating to formaldehyde detoxification; hematopoietic health and cell production from bone marrow is important endpoint"

The paper is also included in Table F-13 "Mechanistic studies relating to lymphohematopoietic cancers, focusing on genotoxicity" in a subsection of the table labelled "Modeling, Endogenous Formaldehyde, and Other Studies" where it is described as "Possibly impactful" with the same description as above.

As mentioned previously, genotoxicity assays in the nasal tissue are negative in rats exposed up to 15 ppm. At these levels, the adducts might be increased ~5-fold above background in the presence of increased cell proliferation. Even conceding that these genotoxicity studies in the nasal tissue may have limitations, they are generally consistent with the studies in ADH5 null mice indicating that more than a 5-fold increase in formaldehyde-DNA adducts are likely needed to induce genotoxicity. Furthermore, genotoxicity studies in the nasal tissue and the studies with ADH5 null mice support the implications of the biologically based dose response (BBDR) model describing nasal tumor formation in rats published nearly two decades ago (Conolly et al. 2003; 2004). Therein, the BBDR model results indicated that there was no requirement for a direct genotoxic component in the model to fit the tumor data as the tumor data could be explained by the increase in spontaneous mutations arising from prolonged increases in regenerative cell proliferation.

Finally, dual isotope formaldehyde inhalation studies indicate no exogenous formaldehyde-DNA adducts can be detected in rat nasal tissue at exposures ≤0.3 ppm (Leng et al. 2019) and that exposure to 0.7 ppm results in exogenous adducts an order of magnitude below endogenous adduct levels (Lu et al., 2011). These results further establish thresholds below 2 ppm.

Individually, the studies described above may not paint a coherent MOA for formaldehyde. In totality, however, they tell a remarkably cohesive picture of the likely MOA for formaldehyde-induce nasal tumors. Instead of integrating these data, the U.S. EPA documents are full of speculative discussions of what might theoretically be capable of happening as evidenced by their refusal to develop either a linear or non-linear MOA and instead display networks of plausible mechanistic effects. If there is a study or studies that report that formaldehyde causes, for example, inflammation in the portal of entry and/or systemic tissues, then EPA accepts these effects at face value and further hypothesizes that they could play a meaningful role in an otherwise overly complex intractable MOA while ignoring high-quality molecular data that tell a remarkably coherent study. This is not how risk assessment should be conducted. The next comment underscores broader concerns about the approach the U.S. EPA have taken in this assessment.

3. U.S. EPA has seemingly abandoned existing frameworks and guidance on conducting MOA analysis.

The U.S. EPA Guidelines for Carcinogen Risk Assessment describes the process of MOA analysis (U.S. EPA 2005). This framework is similar to other existing MOA frameworks as well as AOP frameworks in that they generally describe MOA as a series of key mechanistic events linked by dose-response relationships. In Table 34 of the *Assessment Overview*, EPA lists a series of mechanistic events. Unlike existing MOA and AOP frameworks that describe MOA as a series of key events linked by dose-response relationships, EPA simply lists events with no obvious relationships beginning from exposure to tumor development. For example, the table lists "oxidative stress, immune disease and dysfunction in the URT" as a hypothesized mechanistic event. Notwithstanding that these are not an obvious single mechanistic event, it is unclear how and where these events fall within a sequence of key events leading from exposure to nasal tumors.

Importantly, it appears that EPA is unconcerned with establishing a sequence of events that explain the nasal tumors in rats and then exploring the human relevance of that MOA. Rather, they are adopting an anti-MOA approach. Figures 1-25 and 1-27 cite Smith et al. (2016) in the figure legend. Smith et al. (2016) is not a formaldehyde study, but rather an article that coopts earlier work on the characteristics of cancer (Hanahan and Weinberg 2011) into 10 key characteristics of carcinogens (KCC) which basically attempts to do away with MOA analysis and instead argue that carcinogens act by numerous simultaneous processes. This approach seems to make MOA analysis intractable and quantitative risk assessment unnecessarily complicated. Smith et al. (2016) suggest that the 10 KCC approach "should introduce objectivity that could reduce reliance on expert opinion, as well as facilitate comparisons across agents. . .[and]. . .may afford a broad consideration of the mechanistic evidence rather than focusing narrowly on independent mechanistic hypotheses or pathways in isolation." More recently, Smith et al. (2020) state that the KCC approach is "in contrast to more narrow, reductionist

approaches such as adverse outcome pathway and MOA frameworks that focus on singular events".

One inherent drawback to the above approach is that without some attempt to place key events into an ordered sequence, derivation of toxicity values based on key events occurring before tumor formation becomes increasingly difficult as there is less ability to identify exposure levels that would not likely progress to cancer. This is also antithetical to U.S. EPA (2005) guidance specifying a preference for the use of BBDR models when available. Such models are generally "reductionist" and constructed on critical sub-models that relate exposure to an initiating event that subsequently has a relationship to another key event in a sequence of key events leading to a specific tumor of interest. This reviewer is unaware of any method to relate exposure to multiple KCCs in a manner that is then able to quantitatively predict a specific tumor outcome. Interestingly, the BBDR model for formaldehyde includes both directly mutagenic and cell proliferation-based components in the model, which the modeling results ultimately indicate there is no requirement for a direct mutagenic component as the tumor incidence in rats can be predicted based in increases in spontaneous mutations arising from increased cell proliferation. Notably, the U.S. EPA discounts this model-based insight despite empirical data and mechanistic data that support the model's indication that a direct mutagenic component is unnecessary. Moreover, the U.S. EPA also criticizes some of the BBDR model input parameters. Development of a BBDR based on the MOA diagrams shown in Figures 1-125, 1-126, and 126, if even possible, would be increasingly complex and the parameterization of the various input parameters endlessly debated.

The adoption of the KCC approach by the U.S. EPA indicates that risk assessment is, depending on one's point of view, (d)evolving into an anti-hypothesis, anti-expert, anti-MOA approach of simply demonstrating that a chemical has certain characteristics in common with other carcinogens as opposed to understanding how a chemical causes a specific tumor. It is noteworthy that lead author on the KCC approach (Smith et al. 2016; Smith et al. 2020) is also a coauthor on epidemiological studies linking inhaled formaldehyde exposure to leukemia (Zhang et al. 2010; Zhang et al. 2009).

Instead of using the KCC approach, the U.S. EPA should follow its own guidance for the risk assessment of carcinogens as described in U.S. EPA (2005).

4. U.S. EPA failed to respond to the spirit of the NRC's comments on U.S. EPA's 2010 draft formaldehyde assessment as it relates to toxicokinetics and MOA analysis.

Appendix D includes responses to several comments made in the 2011 NRC External Peer Review of U.S. EPA's 2010 Draft assessment on formaldehyde. In a section on Toxicokinetics, the NRC makes two critical comments. The first (page D-6) is that the committee concludes that the available data at that time supported the hypothesis that inhaled formaldehyde is not delivered systemically (at least up to 15 ppm). The second (page D-8) is that the committee acknowledged that systemic delivery is not a prerequisite for some systemic effects and that indirect MOAs associated with local

irritation, inflammation and stress might be operative. In the disposition of these comments, the U.S. EPA agreed that the lack of systemic delivery is sufficiently supported and that effects such as irritation, inflammation, and oxidative stress may be relevant for MOAs at distal sites.

A reasonable interpretation of the NRC comments is that *some* systemic effects *might* be due to indirect effects such as irritation, inflammation, and oxidative stress, and that without systemic delivery of formaldehyde, evidence for involvement of indirect mechanisms in systemic effects should be demonstrated. In contrast, the U.S. EPA appears to have interpreted the NRC's statement about the possibility of indirect MOAs as permission to speculate/hypothesize that *any* systemic effect could be due to one or all these indirect mechanisms and thus there is no burden on the U.S. EPA to provide strong evidence supporting indirect mechanisms for any of the systemic effects of interest.

It could be argued that once a chemical is determined to not distribute systemically the number of plausible MOAs diminish and the strength of evidence needed to support local exposure causing systemic effects should increase—especially for health effects where the database is comprised of studies scored with medium or low confidence. In essence, the U.S. EPA acceded the NRC's encouragement to accept the strong evidence supporting lack of systemic delivery for formaldehyde and traded it for NRC's acknowledgement that local effects might play a role in some systemic effects. This was then used to support speculation that local effects are somehow responsible for the diverse array of outcomes such as leukemia, reproductive toxicity, developmental toxicity, etc. If generic effects like irritation, inflammation, and oxidative stress were associated with these health effects then it seems likely that more might be known about such MOAs and moreover, that these adverse effects/diseases would be strongly correlated with one another as they must share some common MOA irrespective of formaldehyde exposure. It is anticipated that the NRC will address EPA's new approach in their forthcoming review.

5. The U.S. EPA failed to clearly define the term genotoxicity.

Although DNA modifications can be pro-genotoxic, their presence in DNA is ubiquitous and are not necessarily indicative of heritable gene or chromosomal mutations. The U.S. EPA failed to clearly define the term genotoxicity; however, it is apparent from Table 1-38 "Concordance of temporal and dose-response relationships among formaldehyde effects induced in F344 rat nasal epithelium in vivo" of the *Toxicological Review* that DNA-protein crosslinks and formaldehyde-DNA adducts are considered by the U.S. EPA as genotoxicity. Notably, there are no OECD or OPPTS guidelines to assess genotoxicity based on measurements of adducts.

The U.S. EPA should revise its assessment such that the presence of DNA-protein crosslinks and formaldehyde-DNA adducts is not synonymous with genotoxicity.

6. Unlike U.S. EPA, genotoxicity experts expect systemic delivery of genotoxicants for genotoxicity to occur.

In support of leukemia and genotoxicity, the U.S. EPA acknowledges the lack of systemic delivery but then invokes non-specific local mechanisms causing systemic genotoxicity. These views are in direct contrast to other organizations. For example, the OECD test guideline 474 for *in vivo* micronucleus induction requires proof of systemic delivery of a test article when negative genotoxicity results are to be accepted for regulatory purposes[3]. This implies that the genotoxicity experts who designed the test guidelines anticipate that genotoxicity in distal tissues *requires* systemic delivery of the test article. The doses in these guideline studies typically include a maximal tolerated dose that would presumably elicit local effects that would, if capable of inducing systemic genotoxicity, interfere with the interpretation of the test results. Since indirect genotoxicity mechanisms are not accounted for in guideline tests, it can be surmised that genotoxicity experts do not consider local effects a capable or meaningful contributor to systemic genotoxicity. Additionally, the purpose of genotoxicity testing guidelines and As acknowledged by the NRC and U.S. EPA, inhaled formaldehyde is not systemically distributed up to 15 ppm. As such, the likelihood that local effects are inducing systemic genotoxicity is low. Data from ADH5 null mice (see above) indicate that even 2-fold increases in serum formaldehyde levels do not cause genotoxicity. Given EPA's hypothesis that elevated formaldehyde at the portal of entry might cause inflammation or oxidative stress, one might assume that ADH5 null mice, having elevated formaldehyde in all tissues, would have higher levels of oxidative stress and DNA damage, yet this does not appear to be the case.

7. Mode of Action for Cancer

7.1 The nasal tumor MOA information provided in the *Toxicological Overview* is neither clear nor well-constructed.

Section 4.2.3. *Mode of action information*, of the *Toxicological Overview* is vague and provides no graphical depiction of the hypothesized MOA. Table 34 lists "hypothesized mechanistic events" with no clear indication of the sequence of events. The first mechanistic event is "direct, or presumed direct, genotoxicity and mutagenicity". The reviewer assumes that U.S. EPA has chosen the word 'presumed direct' because much of the evidence for this key event in animals is based on markers of exposure (e.g., DNA adducts and DNA-protein crosslinks) as opposed to markers of effect (e.g., evidence of clastogenic or mutagenic damage).

It is curious that the only real evidence for "direct" genotoxicity and mutagenicity comes from worker and student studies. The use of the term "direct" is highly misleading, as

[3] Although proof of systemic delivery is not required when statistically significant increases in genotoxicity are observed in these studies, this is because the test article is usually administered up to maximally tolerated doses under controlled experimental conditions.

these are not controlled experiments and the workers and students were likely exposed to other agents (chemical or physical) that might increase micronuclei. Notable omissions from Table 35 are Speit et al. (2007) and Zeller et al. (2011). Speit et al. (2007) exposed 21 subjects to formaldehyde for 10 consecutive workdays at exposures ranging from 0.15 to 0.5 ppm for 4 h/day with four 15-min peak exposures to 1 ppm. No statistically significant increases in buccal micronuclei were observed immediately after exposure, or 7 to 21days thereafter. Zeller et al. (2011) exposed 41 male volunteers to formaldehyde in chambers for 4 h/day for 5 consecutive days. Exposures ranged between 0.3 to 0.7 ppm with intermittent peaks of 0.8 ppm. No increases in micronuclei or sister chromatic exchange were observed in nasal tissue or peripheral blood cells immediately after exposure or at several follow up timepoints. It is unclear why these controlled human studies were omitted form Table 35.

Table 35 also lists as indirect support for genotoxicity "strong and consistent evidence of mutagenicity (increased incidence of MN, CA, and chromosomal aneuploidies) in PBLs of human workers". Elsewhere in the assessment, U.S. EPA acknowledges that inhaled formaldehyde does not distribute beyond the site of contact. As such, U.S. EPA must believe that these data indicate that there is strong and consistent evidence that low levels of inhaled formaldehyde are potent enough to induce systemic genetic damage through indirect mechanisms. Given that many scientists might find this difficult to believe, it is incumbent on the U.S. EPA to strengthen their position. Instead, EPA's primary defense appears to be that publish studies reporting a linkage between inhalation exposure and genotoxicity exist and therefore must be true irrespective of other mechanistic data. However, U.S. EPA does little to bolster their conclusions. For example, there is no attempt to evaluate their conclusions vis-à-vis the controlled studies by Speit et al. (2007) and Zeller et al. (2011). U.S. EPA could bolster their conclusions by identifying other chemicals with strong experimental data demonstrating that inhalation exposure results in systemic genotoxicity without systemic delivery of the parent compound or metabolites; however, no such discussion was found. Is formaldehyde the only chemical that exhibits this behavior? If so, why? If the data appear inconsistent with other observations (e.g., lack of systemic deliver), then perhaps the study conclusions (by U.S. EPA or the study authors) need reconsideration. One could simply ask if U.S. EPA omitted this line of "indirect support" would it fundamentally alter their conclusion about the evidence for genotoxicity at the site of contact. I suspect the answer is no; however, it is U.S. EPA's conclusion that the data support systemic genotoxicity without critical comparison to counterfactual data or providing examples of chemicals that exert the same characteristics that calls into question the validity of many of the conclusions in the assessment.

Table 35 lists "cellular mitogenesis in the absence of cytotoxic tissue pathology" as another hypothesized mechanistic event. In the column titled "experimental evidence pertinent to mechanistic event" there is a bullet stating that there is clear evidence for increased URT "cell proliferation under conditions also resulting in tissue pathology" at $\geq$4 mg/m^3. Unless this bullet includes a typo, it is unclear how that bullet supports the hypothesized event. The next bullet states that there is "suggestive evidence" for increased URT "cell proliferation under conditions not clearly causing tissue pathology" <4 mg/m^3. This bullet does support the hypothesized key event; however, note the overall

weak terminology such as "suggestive evidence" of cell proliferation "not clearly causing" pathology. This language is weak and its purpose seems to be to establish cell proliferation at exposure concentrations more relevant to human exposure levels than the higher concentrations clearly associated with increasing cell proliferation and tumors in rodents.

7.2 The nasal tumor MOA information provided in the *Toxicological Review* is neither clear nor well-constructed.

Page 1-302 states that formaldehyde has been shown to be genotoxic or mutagenic in a variety on *in silico* and *in vitro* test systems. To what is EPA referring regarding *in silico* test systems? A search of the *Toxicological Review* for the term "silico" revealed no additional hits. A search of the *Supplemental Information* for the term "silico" revealed one hit in a citation for Yoo & Ito (2018a). Searching for "Yoo" revealed the study in Table F-12 "Mechanistic studies relating to respiratory tract cancers, focusing on genotoxicity additional hits" where the study was listed as "not impactful." As was discussed in Thompson et al. (2020), BBDR *in silico* modeling suggests that formaldehyde-induced nasal tumors in rats could be predicted with little or no direct mutagenic contribution from DNA-protein crosslinks as the nasal tumors could be explained by mutations accumulating from increased regenerative cell proliferation (Conolly et al. 2003).

Assuming the term "in silico" on page 1-302 was an error and that U.S. EPA instead meant "in vivo" test systems have shown formaldehyde to induce genotoxic or mutagenic effects, this statement too is misleading. As discussed in Thompson et al. (2020), genotoxicity assays conducted in the nasal tissues of rats have reported negative findings for clastogenic and mutagenic markers. As already discussed, ADH5 null mice do not exhibit increases in markers of genotoxicity unless other genes involved in formaldehyde detoxification of DNA repair are also knocked out.

Table A-22 "Summary of in vivo genotoxicity studies of formaldehyde inhalation exposure in experimental animals" is highly misleading regarding the evidence for *in vivo* genotoxicity in regions where tumors occur. A total of five studies is listed in the table section "Mutations." Two of the 5 studies are based on the same underlying bioassay where rats were exposed for 2 years (Recio et al. 1992, Wolf et al. 1995). These studies looked for p53 mutations in tumor tissue, which is not a valid approach for assessing whether genotoxicity is an early initiating event in the MOA. Another study, Kitaeva et al. (1990) is in Russian and appears to have reported genotoxicity in bone marrow cells even though the highest exposure concentration was 1.5 mg/m^3, which is not even carcinogenic to the nasal passages of rodents. The fourth positive study is Liu et al. (2009), where male mice were exposed to 0, 2, 20, or 200 mg/m^3 formaldehyde for 2 hours and then mated six weeks later. Exposure to 200 mg/m^3 was reported to increase heritable mutations in offspring. It should be appreciated that the RD_{50} for mice is ~5 mg/m^3 (see Table A-16 in the *Supplemental Information*). It is conceivable that exposure to 200 mg/m^3 would have depressed respiration much further. Data presented in section

A.3 discusses that this reflex bradypnea induces hypothermia in mice (decreasing body temperature by as much as 14 degrees). In addition, U.S. EPA states that reflex bradypnea "also results in decreased blood pO_2 and pCO_2 and increased blood pH." It is well known that hypothermia can cause genotoxicity (Tweats et al. 2007). This has no relevance to humans because exposure to irritant gases does not result in extreme levels of heat loss due to the much lower surface area to volume ratio of large mammals compared to small mammals (Gordon et al. 2008). The fifth study in this section, Meng et al. (2010) did not find increases in mutant frequency in nasal tissue following exposure up to 18.5 mg/m^3 for 13 weeks. This is the only relevant study in this section of the table.

Table A-22 section "Micronucleus" lists only one study reporting on micronuclei in tissue other than blood or bone marrow. Specifically, Neuss et al. (2010) is listed as negative for micronucleus formation in bronchioalveolar lavage cells. Missing from the table is Speit et al. (2011), which reported no increases in micronucleus formation in rat nasal tissue following 4 weeks of exposure up to ~18 mg/m^3 formaldehyde. It is unclear why this study was not included in the table. The vast majority of studies listed in Table A-22 includes either genotoxicity effects beyond the portal of entry or DNA modifications in the nasal and other tissues. These modifications are not measures of heritable gene changes, and thus should not be considered genotoxicity. As already discussed, data in ADH5 null mice indicate that increased formaldehyde DNA adducts are not necessarily genotoxic.

In a subsection of 1.2.5 on page 1-303 it is stated that "long-term occupational exposure was associated with significantly increased MN in PBLs, and aneugenicity appears to be the predominant effect in peripheral tissues (see Section 1.3.3)." There are three problems in this one sentence. First, the U.S. EPA is referring to information supporting URT carcinogenicity that has not yet been discussed. This makes following their arguments unnecessarily difficult. Second, why would formaldehyde genotoxicity be associated with long-term exposure but not shorter-term exposure? Formaldehyde does not bioaccumulate and data indicate that formaldehyde-related adducts are labile. Similarly, if the damage were due to oxidative stress or inflammation, these too are transient effects that likely resolve. Skipping ahead to section 1.3.3 I found no explanation; moreover, if there is an explanation in the document it should be recapitulated whenever such data are used to support MOA. The third issue with the above quote is that U.S. EPA states that aneugenicity appears to be the predominant form of genotoxicity in peripheral blood lymphocytes. Considering that aneugenicity is broadly regarded as a threshold form of genotoxicity, it is curious why low-dose linear extrapolation was conducted. Again, I skipped ahead to section 1.3.3 but found no discussion on the implications of aneugenicity for low dose extrapolation. However, on page 1-524 U.S. EPA concludes that formaldehyde cause aneuploidy in PBLs and clastogenicity in nasal tissues. Notably, Speit et al. (2011) reported that formaldehyde induced clastogenicity but not aneugenicity in cultured cells. Taken together, the reported associations between formaldehyde inhalation exposure and aneugenicity in worker PBLs suggests that such observations are due to something other than formaldehyde exposure.

Much of the text on pages 1-315 to 1-320 includes discussion about the accumulation of DNA-protein crosslinks. This accumulation is discussed in the context of Leng et al. (2019) who reported that exposure to ≤0.3 ppm formaldehyde for 28 days did not result in the detection of exogenous formaldehyde-DNA adducts. U.S. EPA appears to be speculating that longer exposure durations to low concentrations might increase adducts. If accumulation were true, then one might expect that the constant biological production of endogenous formaldehyde would increase the levels of endogenous adducts over time. I am not aware of any articles that have demonstrated this. Considering that U.S EPA believes formaldehyde adducts are pro-mutagenic but also acknowledge that nasal tumors in rodents are exceedingly rare, it would seem that endogenous adducts do not accumulate over time and nor would any exogenous adducts formed from exposure to ≤0.3 ppm formaldehyde accumulate over time. The U.S. EPA also fails to consider that evidence for accumulation of exogenous adducts over time might be due, in part, to remodeling to squamous epithelium where the superficial cell layers are dead yet likely accumulate exogenous adducts as exposure duration increases.

In discussing the role of cell proliferation, U.S. EPA cites evidence for increased cell proliferation ≤2.5 mg/m^3 as evidence for mitogenic proliferation that might increase cancer risk together with DNA damage. As already noted, these levels can result in squamous metaplasia, i.e., a transition from respiratory to squamous epithelium. As such, comparing the labelling index between treated and control animals is essentially comparing the proliferation rate in two different epithelial types.

On page 1-331, U.S. EPA states that glutaraldehyde cause many of the same effects as formaldehyde (squamous metaplasia, hyperplasia, and inflammation) yet does not increase nasal tumors. U.S. EPA uses this to conclude that increased cell proliferation alone is not sufficient to cause nasal tumors. Beyond this one page, the carcinogenicity of glutaraldehyde is not discussed further. The only citation to a research study relevant to glutaraldehyde is Hester et al. (2005), which compared the effects of a single concentration of formaldehyde and glutaraldehyde after instillation (not inhalation). The only other citation relevant to glutaraldehyde was a MOA review by McGregor et al. (2006), which states that the effects "of inhaled glutaraldehyde have not been as extensively studied as those of formaldehyde." To my knowledge, the MOA of glutaraldehyde has not been studied as extensively (if at all) as formaldehyde since 2006. As such, the database for glutaraldehyde is insufficient to inform the MOA of formaldehyde.

On page 1-332 U.S. EPA states that there is sufficient evidence that formaldehyde causes URT carcinogenicity by "at least two primary mechanisms: genotoxicity-associated mutagenicity and cytotoxicity-induced regenerative hyperplasia." I assume that the former mechanism involves direct genotoxicity related to adduct formation, although the terminology is awkward. Also, the "at least two primary mechanisms" phrase is troubling because preceding sections discusses several "other factors modifying the MOA" that are presumably not *primary* mechanisms. Given the extensive research on formaldehyde, it is not clear why the equivocation is necessary. More importantly, if U.S. EPA believes that these are the two primary drivers of URT carcinogenicity and there is broad scientific

consensus that there are exposures that do not increase proliferation and exposures that do not increase exogenous adducts, then it is perplexing that one of these apparently threshold mechanisms could not serve as a basis for an RfC protective against URT carcinogenicity. The application of uncertainty factors could account for any remaining uncertainty U.S. EPA has about where these thresholds occur in humans. Instead, U.S. EPA appears to accept every publication equally to the point of decision paralysis about whether there is any lower bound on exposures that do not result in proliferation or mutation-inducing adducts and therefore U.S. EPA defaults to linear no threshold cancer slope factor development. The term decision paralysis is generous, as one migt also conclude that U.S. EPA is outright reluctant to conduct a non-linear assessment on formaldehyde even though it is a chemical the body generates, detoxifies, and utilizes every day.

8. Data supporting histopathological changes in the human upper respiratory tract (URT) is weak.

On page 1-154 of the *Toxicological Review*, EPA states that most of the studies reporting histopathological changes in humans had average exposures ranging from 0.05 to 0.6 mg/m^3. EPA speculates that these workers are likely less sensitive to formaldehyde than the average person, characterizing them as "survivors" of long-term irritant exposure. This appears to be complete speculation and, to this reviewer, undermines the credibility of the assessment. Notably, there were no high confidence studies in humans. Among the four medium confidence studies, one (Boysen et al. 1990) was characterized by EPA as equivocal. Importantly, all the studies found relatively mild differences in nasal histopathology when plant workers were compared to non-plant workers (see table below). Many of the plant workers were exposed to wood dust and likely other chemicals. As shown in the table below, many of the individuals in the referent groups appear to work in very different professions with different environmental settings (e.g., factory vs hospital). These comparison groups do not appear to be suitable for ascribing any observed differences to formaldehyde with any certainty.

Table. Summary of Comparison Groups for Human URT Histopathology Studies

Study	**Exposed Group**	**Referent Group**
Ballarin et al. (1992)	Plywood factory workers	University or hospital clerks
Boysen et al. (1990) (EPA considered the results equivocal)	Chemical company producing formaldehyde	Office staff at chemical company, hospital laboratory personnel, outpatients at the ear, nose, and throat department of a hospital
Holmstrom et al. (1989); Holmstrom & Wilhelmsson (1988)	Formaldehyde production workers; workers exposed to wood dust and formaldehyde	Persons from the local government with no history of formaldehyde exposure
Edling et al. (1988, 1987)	Workers from particle board	Men with similar smoking

	and laminae-processing	habits and no known industrial exposure to formaldehyde

Adapted from Table 1-25 in the Toxicological Review.

9. The benchmark dose modeling conducted in this assessment is nearly a decade old and uses an unsupported version of the BMD modeling software

It is difficult to verify all the BMD modeling results as Appendix B clearly indicates that the model outputs were run nearly a decade ago in January of 2013, apparently using EPA's BMD software (BMDS) v1.9. The current version of BMDS is v3.2. U.S. EPA's BMDS website (https://www.epa.gov/bmds) explicitly states that BMDS 2.7 is no longer supported, and this must also be true for prior versions. It is reasonable to expect that any new U.S. EPA assessment should use the most current (and supported) version of BMDS. The U.S. EPA should re-run all the BMD modeling in the assessment with the latest version of BMDS.

Curiously, many of the BMD modeling results in this document are expressed in units of mg/kg-d as opposed to mg/m^3. This is likely a result of the modeling software not knowing the units, but it is surprising that EPA did not correctly indicate the units. Moreover, all the discussion about BMD models should technically be benchmark concentration (BMC) as these are inhalation concentrations as opposed to oral doses.

10. U.S. EPA's benchmark dose modeling of rat respiratory histopathology has several reporting and methodological flaws.

Table B-9 in *Supplemental Information* contains a column labelled "Model fit" that points the reader to the model plot for that particular BMD model. The column refers to Fig. 3, Fig. 4, Fig. 7, and Fig. 8; however, there are no such figures. It seems that the column should instead point to Figures B-9 to B-12. Figures B-8, B-9, and B-10 are poorly labelled, as they do not indicate which study is being modeled.

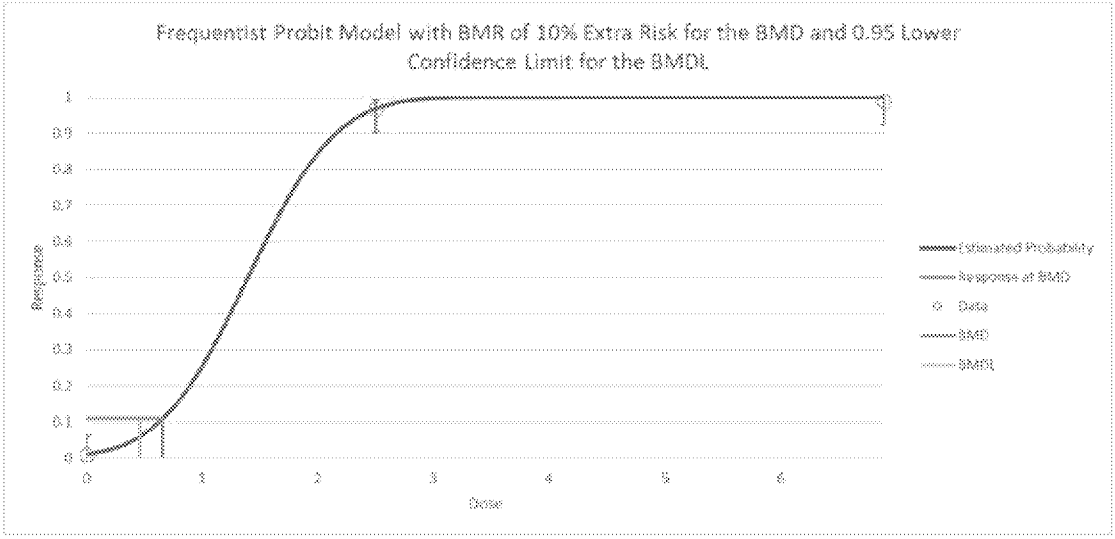
Table B-9 indicates that the U.S. EPA selected the log-logistic model for the 28-month squamous metaplasia data in Woutersen et al. (1989). The BMD and BMDL values for this model match the values listed in Table 24 of the *Assessment Overview*. In contrast, the BMD and BMDL for the log-probit model for squamous metaplasia in Kerns et al. (1983) in Table B-9 (0.576 and 0.448 mg/m^3) ***do not match*** the BMD and BMDL listed for this study in Table 24 of the *Assessment Overview* (0.587 and 0.456 mg/m^3). These are minor discrepancies, but they should presumably match.

Similarly, the human POD for the Woutersen et al. (1989) study in Table 24 (0.94 mg/m^3) matches the PODs in Table 25, whereas the POD for Kerns et al. (1983) in Table 24 (0.086 mg/m^3) does not match the corresponding POD in Table 26 (0.88 mg/m^3). Again, these are minor discrepancies but should presumably match as one progresses

through the assessment. Based on Tables 2-5 and 2-6 in the *Toxicological Review*, it appears that the correct BMDL (or BMCL) is 0.448 mg/m^3 and the correct human POD is 0.086 mg/m^3. These discrepancies across documents and tables need to be rectified in further drafts of the IRIS review of formaldehyde.

In developing a POD for squamous metaplasia from Kerns et al. (1983) the U.S. EPA used the 18-month data because the 24-month data resulted in poor model fits. Because of this, the U.S. EPA included a 3-fold uncertainty factor for the use of less than chronic data (UF_S; see Table 25 in the *Assessment Overview*). However, U.S. EPA BMD modeling guidance allows for the omission of high dose groups to improve model fit, especially when the response level has plateaued (U.S. EPA 2012). Using BMDS v3.2, the 24-month data could be fit after omitting the highest dose (see Figure below). In doing so, the BMC and BMCL were 0.66 and 0.46 mg/m^3, respectively. These values are nearly identical to the values derived from the 18-month data. Using these BMD and BMDL values from the chronic data obviates the need to include the 3-fold UF_S that EPA applied.

According to text on page B-18 of the *Supplemental Information*, the flux associated with 0.488 mg/m^3 is 685 pmol/mm^2-hr[4]. Considering that both 0.488 mg/m^3 (18-month data) and 0.46 mg/m^3 (24-month data) round to 0.5 mg/m^3, it can be estimated that the 24-month data results in the same flux and same human equivalent dose as the 18-month data. As such, the RfC U.S. EPA derived from the Kerns et al. (1983) 18-month data of 0.0009 mg/m^3 (0.088 mg/m^3 ÷ a 100-fold composite uncertainty factor, UF_C) would be 3-fold higher using the 24-month data due to the omission of the 3-fold UF_S, i.e. 0.003 mg/m^3 (0.088 mg/m^3 ÷ 30). Notably, this matches the candidate RfC EPA derived from the Woutersen (1989) study.



[4] Note: there is a typographical error in the calculation at the end of the first paragraph on page B-18, where "1528.18 x 0.488-685 pmol/mm^2-hr" should read "1528.18 x 0.488 = 685 pmol/mm^2-hr"

Figure. BMD modeling results for squamous metaplasia rats following exposure to formaldehyde for 24 months. Note: the highest study dose was dropped to improve model fits, consistent with U.S. EPA (2002) BMD modeling guidance. Source: U.S. EPA (2022) Table 1-26 of *Toxicological Review*.

11. U.S. EPA's benchmark dose modeling of male reproductive toxicity studies has several potential flaws.

Table 28 in the *Assessment Overview* lists eligible studies for RfC derivation based on reproductive and developmental toxicity. Therein, Ozen et al. (2002) is listed as the basis for a point of departure derived from changes in relative testes weight. In the subsequent related tables (Table 30 & 31), this study is erroneously referred to as Ozen et al. (2005). Data for this endpoint is contained in a separate document, the *Toxicological Review*, where in Table 1-57 results from Ozen et al. (2002) are summarized by EPA, reporting the % decrease in relative testes weight. Although these are marked as statistically significant, the reductions never exceeded 10%. In fact, 2 and 3% reductions are listed as statistically significant. Since these data were carried forward for RfC derivation, it is surprising that the actual underlying data are not summarized in the tables of the *Toxicological Review* or *Assessment Overview*. Instead, the data are included in the *Supplemental Information*.

As stated on page B-26 in the *Supplemental Information*, the customary benchmark response (BMR) for changes in body and organ weight is 10%. However, when using this BMR, the BMD modeling results yielded a BMD_{10} value *above* the range of observation for the 4-week time point, meaning that the customary effect size was not observed in Ozen et al. (2002), but rather would be estimated to occur at *higher* exposure concentrations than employed in the study. The U.S. EPA considered this "unacceptable extrapolation" and therefore elected to instead use a BMR of 1 standard deviation (1SD). Using this BMR, the $BMDL_{1SD}$ was *below* the range of observation. It is important to recognize that the mean relative testes weight in the control, low dose, and high dose groups were 0.94, 0.92, and 0.91 (i.e., very similar). The same issue occurred with the 13-week data, where again the BMD_{10} was estimated to fall *above* the range of observation and thus EPA opted for a BMR of 1SD.

The Figure below shows BMD modeling results for the 13-wk testes data using the 10% and 1SD BMRs using BMDS v3.2. As can be seen in both plots, there is little to no apparent dose-response. Notably, the BMD_{1SD} was estimated to be 0.19 mg/m^3; however, because of the extrapolation below the range of observation, the U.S. EPA used a NOAEL/LOAEL approach and identified the lowest concentration of 2.93 mg/m^3 as the LOAEL and subsequently applied a 10-fold uncertainty factor for the absence of a NOAEL thereby effectively making this value similar to the $BMDL_{1SD}$ of 0.19 mg/m^3.

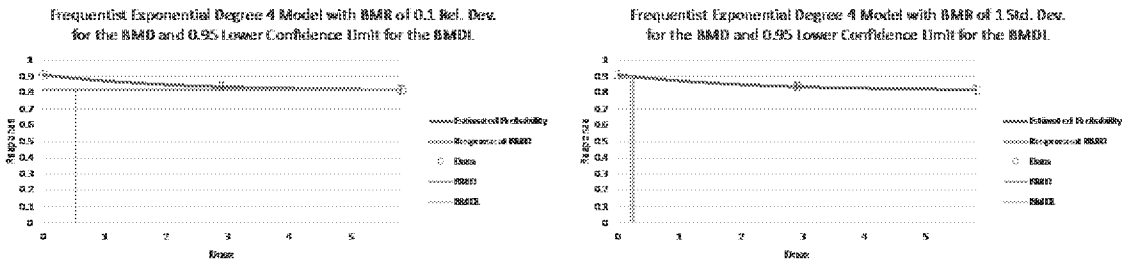


Figure. Comparison of BMD modeling results of changes in relative testes weight in rats following exposure to formaldehyde. Left: Using the customary BMR of 10% for change in body or organ weight, the best model estimates that the 10% response occurs at an exposure concentration *higher* than used in the study (i.e., the green vertical BMD line is not evident). Right: Using a BMR of 1SD, the best model estimates that the 1SD response occurs at an exposure concentration *much lower* than used in the study (although this model fit the data, the BMD software included warnings about the BMD and BMDL being several fold below the lowest study dose. Note the study concentrations of 5 and 10 ppm were duration adjusted to continuous exposure before modeling, consistent with U.S. EPA's approach. Source: Ozen et al. (2002).

Ozen et al. (2002) did not present the testes or bodyweight data, rather they presented the testes weight as a percentage of bodyweight. Considering that Ozen et al. (2002) reported treatment related decreases in bodyweight gain of 55.9, 34.7, and 20.1% in the control, low, and high dose, respectively, it is unclear what the biological significance is of changes in testes weight as a percent of bodyweight if treatment is also affecting bodyweight. Considering further that humans are unlikely to be exposed to concentrations of formaldehyde that alter bodyweight (or bodyweight gain), the data are even more uncertain. Given that Ozen et al. (2002) is a very short paper with a single table of biological results it is surprising that EPA gave this a *high* confidence score and carried it forward for RfC derivation.

To further illustrate the uncertainty of Ozen et al. (2002) one might consider the changes in bodyweight gain mentioned above as perhaps more serious. In fact, the Figure below indicates a stronger dose-response for this effect as compared to the testes. The $BMDL_{10}$ for this effect is ~0.6 mg/m^3, and applying the same additional uncertainty factors the EPA applied to the testes POD results in a candidate RfC of 0.002 mg/m^3. The notion that humans would experience effects such as weight loss at 0.002 mg/m^3 is as seemingly unlikely as experiencing reproductive toxicity at concentrations exceeding the candidate RfC value of 0.001 mg/m^3. The following section addresses the bodyweight gain and testes weight data from a MOA perspective.

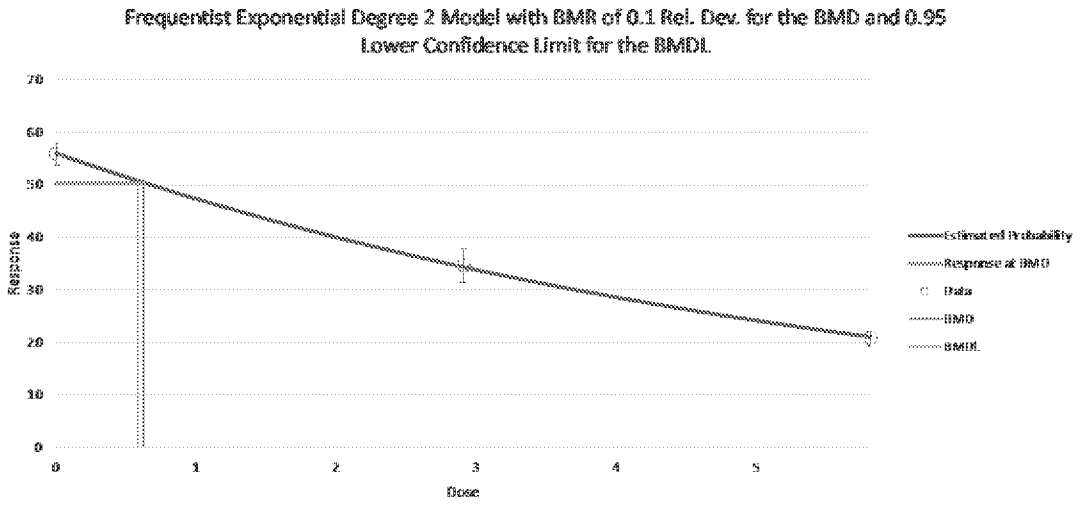


Figure. BMD modeling results of changes in bodyweight gain in rats following exposure to formaldehyde. Note the study concentrations of 5 and 10 ppm were duration adjusted to continuous exposure before modeling, consistent with U.S. EPA's approach. Source: Ozen et al. (2002).

12. The U.S. EPA's hypothesized MOA for male reproductive effects misses a critical alternative explanation.

Modeling the serum testosterone data from Ozen et al. (2005) resulted in good fitting models with a $BMDL_{1SD}$ value of 0.21 mg/m^3 in agreement with U.S. EPA (data not shown). Interestingly, this POD is similar to that derived for bodyweight gain changes and testes (see above). A very quick literature search reveals that food restriction affects testes weight and serum testosterone levels in rats (Rehm et al. 2008). If the bodyweight gain changes reported in Ozen et al. (2002) were due to reduced feed intake, then the changes in testes weight and serum testosterone might be related to changes in bodyweight as opposed to formaldehyde toxicity per *se*. Notably, these effects all occur at about the same concentration—a possible indicator that they are in fact related to one another. This hypothesis seems as likely, if not more likely, than U.S. EPA's hypotheses about indirect mechanisms like inflammation, oxidative stress, and neuroendocrine disruption (see section 1.3.2 of the *Toxicological Review*). That the linkage between feed intake and these same markers of toxicity are not even considered by EPA is problematic. Notably, the Ozen et al. (2002) study that EPA scored as *high* makes no mention of feed or water consumption despite the significant decreases in bodyweight gain. This calls into question the overall thoroughness and quality of this study.

13. An overall comment on the organization of the assessment.

Overall, the document is difficult follow as pertinent information is scattered amongst three separate documents: an *Assessment Overview*, a *Toxicological Review,* and *Supplemental Information*. This reviewer found it difficult to follow the assessment whether reading printed hard copies or a computer screen. In some places there are

lengthy passages without any supporting references, making it difficult for the reader to know which studies the U.S. EPA is using to support a given statement, even for readers familiar with the formaldehyde literature. As written, topics are covered in differing degrees of depth in multiple places. Perhaps an alternative approach would be to have combined the *Assessment Overview* and *Toxicological Review* into two or three separate documents with as minimal overlap as possible. Possible alternatives include portal of entry (POE) vs systemic effects or cancer vs non-cancer effects—perhaps with dose-response analysis in a separate document altogether. The current approach needs serious reconsideration.

Select References

Conolly RB, Kimbell JS, Janszen D, Schlosser PM, Kalisak D, Preston J, Miller FJ. 2003. Biologically motivated computational modeling of formaldehyde carcinogenicity in the f344 rat. Toxicol Sci. 75(2):432-447.

Conolly RB, Kimbell JS, Janszen D, Schlosser PM, Kalisak D, Preston J, Miller FJ. 2004. Human respiratory tract cancer risks of inhaled formaldehyde: Dose-response predictions derived from biologically-motivated computational modeling of a combined rodent and human dataset. Toxicol Sci. 82(1):279-296.

Gordon CJ, Spencer PJ, Hotchkiss J, Miller DB, Hinderliter PM, Pauluhn J. 2008. Thermoregulation and its influence on toxicity assessment. Toxicology. 244(2-3):87-97.

Hanahan D, Weinberg RA. 2011. Hallmarks of cancer: The next generation. Cell. 144(5):646-674.

Leung J, Wei W, Liu L. 2013. S-nitrosoglutathione reductase deficiency increases mutagenesis from alkylation in mouse liver. Carcinogenesis. 34(5):984-989.

Pontel LB, Rosado IV, Burgos-Barragan G, Garaycoechea JI, Yu R, Arends MJ, Chandrasekaran G, Broecker V, Wei W, Liu L et al. 2015. Endogenous formaldehyde is a hematopoietic stem cell genotoxin and metabolic carcinogen. Mol Cell. 60(1):177-188.

Rehm S, White TE, Zahalka EA, Stanislaus DJ, Boyce RW, Wier PJ. 2008. Effects of food restriction on testis and accessory sex glands in maturing rats. Toxicol Pathol. 36(5):687-694.

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, DeMarini DM, Caldwell JC, Kavlock RJ, Lambert PF et al. 2016. Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. Environ Health Perspect. 124(6):713-721.

Smith MT, Guyton KZ, Kleinstreuer N, Borrel A, Cardenas A, Chiu WA, Felsher DW, Gibbons CF, Goodson WH, 3rd, Houck KA et al. 2020. The key characteristics of carcinogens: Relationship to the hallmarks of cancer, relevant biomarkers, and assays to measure them. Cancer Epidemiol Biomarkers Prev. 29(10):1887-1903.

Tweats DJ, Blakey D, Heflich RH, Jacobs A, Jacobsen SD, Morita T, Nohmi T, O'Donovan MR, Sasaki YF, Sofuni T et al. 2007. Report of the iwgt working group on

strategies and interpretation of regulatory in vivo tests i. Increases in micronucleated bone marrow cells in rodents that do not indicate genotoxic hazards. Mutat Res. 627(1):78-91.

U.S. EPA. 2005. Guidelines for carcinogen risk assessment, epa/630/p-03/001f. Risk Assessment Forum: US Environmental Protection Agency, Washington, DC.

U.S. EPA. 2012. Benchmark dose technical guidance. Risk Assessment Forum. EPA/100/R-12/001.

Zhang L, Freeman LE, Nakamura J, Hecht SS, Vandenberg JJ, Smith MT, Sonawane BR. 2010. Formaldehyde and leukemia: Epidemiology, potential mechanisms, and implications for risk assessment. Environ Mol Mutagen. 51(3):181-191.

Zhang L, Steinmaus C, Eastmond DA, Xin XK, Smith MT. 2009. Formaldehyde exposure and leukemia: A new meta-analysis and potential mechanisms. Mutat Res. 681(2-3):150-168.